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Epigenetics in autoimmune disorders:
Highlights of the 10th Sjögren's Syndrome Symposium

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Abstract

During the 10th International Symposium on Sjögren's Syndrome held in Brest, France, from October 1-3, 2009 (<http://www.sjogrensymposium-brest2009.org>), the creation of an international epigenetic autoimmune group has been proposed to establish gold standards and to launch collaborative studies. During this “epigenetics session”, leading experts in the field presented and discussed the most recent developments of this topic in Sjögren's Syndrome research. The “Brest epigenetic task force” was born and has scheduled a meeting in Ljubljana, Slovenia during the 7th Autoimmunity congress in May 2010. The following is a report of that session.

Key-words : Epigenetics, Sjögren's syndrome, rheumatoid arthritis, autoimmune diseases

Take-home messages:

- Epigenetic regulatory mechanisms comprise DNA methylation, a variety of histone modifications, and microRNA activity; all of which act upon gene and protein expression levels.
- Increased activity and destructive potential of synovial fibroblasts in rheumatoid arthritis is due in part to the deregulation of epigenetic factors.
- Development of an epigenetic bank and the establishment of gold standards in an epigenetic consortium to analyse epigenetic modifications are needed.
- Epigenetic alterations in autoimmune diseases could be used as prognostic/diagnostic tools and markers of immune cell activation.

1. Introduction

The mechanisms causing most human autoimmune diseases remain obscure. Although genes and genetic loci causing predispositions to autoimmunity are being identified, incomplete disease concordance in identical twins indicates that non-genetic factors and mechanisms also contribute to autoimmune etiologies. Eukaryotic gene expression requires not only the regulated activity of transcription factors, but also a transcriptionally permissive regional chromatin configuration. The structure of chromatin is regulated by epigenetic mechanisms, primarily DNA methylation and histone modifications. Several recent studies have demonstrated that disrupting the epigenetic regulation of transcription plays crucial roles in the development of autoimmune diseases. The main purpose of the “epigenetics session” at Brest was not only to present recent academic works, but also to obtain a more comprehensive view of the current state of epigenetics research in the international autoimmunity community.

Epigenetic modifications are changes in gene expression that can be maintained throughout a cell’s life and be passed on through cell division, and thus can remain persist in the organism without alterations in DNA sequences. Epigenetic factors are associated with a great number of diseases, including cancer, autoimmune disorders, heart diseases and skin diseases. Since drugs that can reverse aberrant gene expression profiles are readily available, the identification and subsequent modification of epigenetic markers involved in disease development may provide important novel

therapeutic approaches for autoimmune disorders.

2. What are epigenetic modifications?

Epigenetic regulatory mechanisms comprise DNA methylation, a variety of histone modifications, of which the best characterized is acetylation, and microRNA activity; all of which act upon gene and protein expression levels [1-3]. In vertebrates, DNA methylation is defined as the postsynthetic addition of methyl groups to cytosine bases, generally only those in CpG pairs, at position 5 of the pyrimidine ring, and catalyzed by a DNA methyltransferase (DNMT). About 70% of CpG dinucleotides in human DNA are methylated in pairs, while most of the unmethylated CpGs are situated in CpG islands. CpG islands are CG-rich sequences located near coding sequences, and often contain regulatory elements for nearby genes. Approximately half of mammalian gene loci have CpG islands [1]. DNA methylation leads to gene silencing by binding methyl-CpG-binding proteins, such as MeCP2 and MBD2, which then recruit chromatin inactivation complexes containing histone deacetylases (HDACs) and histone methyltransferases (HMTs). Chromatin inactivation complexes promote the condensation of DNA into a transcriptionally restrictive configuration in the regions encompassing methylated sequences. DNA methylation may also interfere with the binding of some transcription factors [2].

Histone modifications regulate gene expression by changing the degree to which gene loci are accessible to transcription machinery. The N-terminal tail of histones protrude

from nucleosomal core particles and are subject to an array of post-translational modifications[3]. These modifications include, but are not limited to, acetylation, methylation, phosphorylation, ADP-ribosylation, sumoylation and ubiquitination.

MicroRNAs (miRNAs) are a group of post-transcriptional regulators involved in many biological processes including development, differentiation, proliferation and apoptosis [4]. miRNAs are ~22nt-long non-coding RNAs that suppress translation by binding to, and thereby causing the degradation of, complementary target mRNA species [5]. Briefly, miRNAs are genome-encoded and transcribed by RNA polymerase II, similar to ordinary protein-coding RNAs. Transcription of a miRNA-coding gene generates a primary transcript, known as pri-miRNA, that contains a polyA tail and 7-methylguanosine cap. The pri-miRNA is processed into a 70-nt stem-loop structure called pre-miRNA by the RNase III enzyme Drosha. Exportin 5 imports the pre-miRNA to the cytoplasm where it is further processed by the RNase III protein Dicer into an unstable 19-25 nt miRNA duplex structure that possesses a 'guide strand' (miR) and a 'passenger strand' (miR*). miR* is degraded and miR becomes the mature miRNA that is incorporated into the RNA-induced silencing complex (RISC) to regulate protein expression [4].

3. Epigenetic modifications and autoimmune diseases

Continued efforts in the field of epigenetics have led to the discovery of associations between every epigenetic modification mentioned above and one or more

autoimmune disorder. Specific epigenetic defects associated with rheumatoid arthritis (RA), systemic lupus erythematosus (SLE), primary Sjögren's syndrome (pSS) and systemic sclerosis (SSc) were presented and are summarized below.

3.1. rheumatoid arthritis

RA is an autoimmune disease characterized by chronic joint inflammation that can cause progressive and irreversible destruction of articular cartilage. Although the cause of RA is unknown, a combination of genetic susceptibility factors, deregulated immunomodulation and environmental influences is generally considered to be at the root of autoimmune responses that occur in synovial joints of RA patients.

In recent years, Steffen Gay (University Hospital Zürich, Switzerland) and his colleagues have pursued the idea that, along with immune cells, synovial fibroblasts residing in the joint space play important roles in the perpetuation, and perhaps even the initiation of RA. Moreover, they have found that the increased activity and destructive potential of RA synovial fibroblasts (RASFs) is due in part to the deregulation of epigenetic factors.

As discussed by Dr. Gay, synovial tissue DNA in RA patients is hypomethylated, and when normal synovial fibroblasts are treated with the DNMT inhibitor 5-AZA (5-aza-2'-deoxycytidine), they develop a RASF-like phenotype [6, 7]. A recently published report also provides evidence of histone hyperacetylation in synovial tissue samples from animals with a RA-like condition compared to animals with

osteoarthritis (OA). Furthermore, the authors find the activity of HDACs to be about two-fold lower in synovial extracts from RA patients compared to OA patients [8]. Lastly, a recent study has suggested that the expression and function of specific miRNAs, possibly miR-155 and miR-146, might also be involved into the pathogenesis of RA [9].

With regards to the involvement of miRNAs in autoimmune disorders, the discussion focused mainly on the role of miRNA-expressing microparticles and their utility as prognostic/diagnostic tools and markers of immune cell activation, or their potential role on target cells.

3.2. Systemic lupus erythematosus

SLE is an autoimmune disorder characterized by the overproduction of auto-antibodies, which ultimately lead to autoimmune responses and tissue damage in multiple organs. Both genetic and environmental factors contribute to the development of the disease, but the specific interplay between immune system and environmental factors that leads to disease in genetically predisposed hosts remains poorly understood.

Several studies from Dr. Lu's group (Epigenetic Research Center, Central South University, China) and others have uncovered the importance of DNA hypomethylation in the etiology of SLE [10, 11]. Specifically, recent evidence

suggests that modulations in DNA methylation due to environmental agents may affect the structure of T cell chromatin, disrupting gene expression and resulting in immune system hyperactivity. The changes in DNA methylation are regulated by the extracellular signal-regulated kinase (ERK) signaling pathway [12], and transgenic mice expressing dominant-negative MEK in T cells display lupus-like T cell auto-activation. Decreased ERK pathway signaling in the T cells of these mice leads to decreased expression of DNMT1 and overexpression of methylation-sensitive autoimmunity genes, such as CD11a and CD70, similar to T cells in human lupus [13]. Demethylating drugs such as 5-azacytidine (5-aza), procainamide and hydralazine can also induce lupus-like autoimmunity, both *in vitro* and *in vivo* [11].

In general, histone modifications appear abnormal in SLE. However, compared with healthy controls, global acetylation of histones H3 and H4 in active lupus CD4⁺ T cells is decreased and H3 acetylation levels negatively correlate with disease activity in lupus patients (as measured by SLEDAI). Global H3K9 hypomethylation is also observed in both active and inactive lupus CD4⁺ T cells compared with controls, whereas H3K4 methylation levels do not differ between patients and controls [14]. The splenocytes of MRL-lpr/lpr mice (which have a lupus-like condition) also exhibit histones H3 and H4 hypoacetylation, although there is an overall global site-specific hypermethylation in these mice compared to controls. These mice also exhibit histone modification defects that have not yet been identified in patients, such as H3K18 methylation, H4K31 methylation, and H4K31 acetylation [15].

Another recent study showed that miR-146a, a negative regulator of the interferon

(IFN) pathway, contributes to the pathogenesis of SLE. By targeting the key signaling components, decreased levels of miR-146a lead to increased type I IFN pathway activity in lupus patients [16]. In addition, Dai et al [17] identified 16 miRNAs that are differentially expressed in SLE.

3.3. Primary Sjögren's syndrome

At the “epigenetics session”, Gabor Illei (NIH, Bethesda, USA) presented his results showing that two miRNAs (miR-574 and miR-768-3p) are overexpressed in the salivary glands of SS patients. He reported that these two suspected epithelial cell miRNAs could be used to predict the evolution of the disease. Seunghee Cha (University of Florida, USA) presented data obtained from her studies of non-obese diabetic (NOD) mice (B6DC) that develop a disease similar to human SS [18]. She highlighted two miRNAs (150 and 146) that are upregulated in target tissues and in PBMCs of B6DC mice compared to control mice. Dr. Cha also reported that miR-146 expression is increased in PBMCs and salivary glands of SS patients.

3.4. Systemic sclerosis

Defective T-cell DNA methylation is also associated with SSc, an autoimmune disorder characterized by vasculopathy and widespread organ fibrosis. CD4⁺ T-cell DNA from patients with SSc is significantly hypomethylated relative to healthy

controls, and the levels of DNMT1, MBD3, and MBD4 mRNAs are significantly decreased in SSc patients [19]. Furthermore, reduced expression of DNMT3a results in enhanced histone acetylation. Fibroblasts synthesize exuberant levels of collagen in SSc and this phenotype is linked to hypermethylation-dependent repression of the collagen suppressor gene FLI1. CpG islands in the FLI1 promoter region are heavily methylated in SSc fibroblasts, as well as in skin biopsy specimens [20]. During the 10th SS Symposium, Carlo Selmi (University of Milan, Italy) presented his work regarding the importance of X chromosome deletion (monosomy X) in Primary biliary cirrhosis (PBC) and scleroderma [21] Dr. Selmi opened a discussion on the importance of conducting a twin studies to uncover the role of epigenetic modifications that affect the expression of X chromosome encoded autoimmune-related genes.

4. Epigenetic consequences

4.1. B and T cell autoreactivity

Yves Renaudineau (University of Brest, France) presented evidence detailing the contribution of DNA demethylation defects to the autoreactivity of B cells [22]. Indeed, testing the influence of IL-6 overproduction by SLE B cells, it was observed that IL-6 abrogates the ability of SLE B cells to induce DNMT1, and then to methylate DNA. This effect is reversed in the presence of a blocking monoclonal

antibody to IL-6 receptor, and reproduced in B cells from healthy controls when the cells are stimulated in the presence of IL-6, or treated with a DNMT1 inhibitor. Such effect has been attributed to an IL-6-dependent cell cycle blockage in B cells allowing overexpression of genes such as human endogenous retrovirus (HERV) and the BCR rearrangement X [23, 24]. Animal models support also that DNA methylation is associated with activation of lymphocytes, leading to the development of an SLE-like disease with autoantibody production. This was demonstrated using DNMTs inhibitors orally administrated or after adoptive transfer using pre-treated CD4⁺ T cells or bone marrow B cells [25].

4.2. Epigenetic protein modifications and autoantibody recognition

Athanasios Tzioufas (University of Athens, Greece) and Stefano Bombardieri (University of Pisa, Italy) reported that histones and other nucleic acid binding proteins can be subject to epigenetic post-translational modifications (acetylation, methylation, phosphorylation, ADP-ribosylation, sumoylation and ubiquitination) [26, 27]. These proteins include autoimmunity-related genes such as Ro60 and Sm, and specific recognition by auto-antibodies may underlie epigenetic modifications in autoimmune diseases. As a consequence it has been proposed that these autoantibodies should be important as tool for diagnosis or as prognostic biomarker.

4.3. New epigenetic therapeutics

Finally, Wesley Brooks (Drug Discovery, Tampa, USA) reported that blocking the human S-Adenosylmethionine (SAM) Decarboxylase with a specific inhibitor prevents the SAM's degradation [28]. As a consequence this new drug should reduce the DNA methylation observed in autoimmune diseases by providing more SAM to the cell. The mechanisms leading to SAM-decarboxylase overexpression in autoimmune diseases were also presented and may be related to the over-expression of the putrecine's pathway (by virus and or as a consequence of the Chromosome X demethylation) [29]

4.4. Establishment of seven epigenetic working groups

At the conclusion of the “epigenetics session”, the participants proposed the creation of an international epigenetic autoimmune group, which would establish gold standards and promote cooperation within the research community.

Seven tentative working groups were formed. These groups aim to pursue the following:

1. Validating and optimizing miRNA markers for autoimmune diseases.
2. Defining HERV markers for autoimmunity.
3. Investigating the role of DNA methylation and related enzymes in autoimmune diseases.
4. Investigating post-translational modifications and auto-antibody recognition. A 3D

bio-computer scientist will likely be required for this group.

5. Investigating the role of X chromosome genes in autoimmunity.

6. Twin studies.

7. Drug discovery related to epigenetic autoimmunity factors.

5. Concluding remarks

The “epigenetics session” of the 10th International Symposium on SS provided an opportunity to relate and discuss the latest research findings in the field of epigenetics. Participants candidly agreed on the creation of a worldwide epigenetic bank and to the development of gold standards in an epigenetic consortium. The momentum in the field is considerable, and all participants look forward to the “epigenetics session” that will take place during the Autoimmunity Congress in Ljubljana, May 5-9, 2010.

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